VECTORS HAVING ENHANCED EXPRESSION, AND METHODS OF MAKING AND USES THEREOF

RELATED APPLICATIONS

Reference is made to the concurrently filed application of Tartaglia et al., Ser. No. 08/815,809. Reference is also made to the copending applications of Paoletti et al., Ser. Nos. 08/417,210, 08/303,275, 08/709,209, 08/184,009 (incorporating by reference Ser. Nos. 07/805,567, from which U.S. Pat. No. 5,378,457 issued) and 08/521,016 and to U.S. Pat. Nos. 5,378,457, 5,225,336, 5,453,364, 5,494,807, 5,505,941, and 5,110,587, all of which patents and applications are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to enhanced vectors, and methods for making and using them. The vectors can have enhanced transcription or translation or enhanced transcription and translation and/or expression, e.g., enhanced transcription or translation or transcription and translation and/or expression from a nucleotide sequence of interest.

Several publications are referenced in this application. Full citation to these publications is found where cited or at the end of the specification, immediately preceding the claims or where the publication is mentioned; and each of these publications is hereby incorporated by reference. These publications relate to the state of the art to which the invention pertains; however, there is no admission that any of these publications is indeed prior art.

BACKGROUND OF THE INVENTION

DNA such as plasmids or naked DNA, and other vectors, such as viral vectors, e.g., vaccinia virus and more recently other poxviruses, have been used for the insertion and expression from foreign genes. The basic technique of inserting foreign genes into live infectious poxvirus involves recombination between pox DNA sequences flanking a foreign genetic element in a donor plasmid and homologous sequences present donor plasmid and homologous sequences present in the rescuing poxvirus (Piccini et al., 1987). Recombinant poxviruses are constructed in steps known as in or analogous to methods in U.S. Pat. Nos. 4,769,330, 4,772,848, 4,603,112, 5,505,941, and 5,494,807, incorporated herein by reference. A desire in vector development is attenuated vectors, e.g., for enhanced safety; for instance, so that the vector may be employed in an immunological or vaccine composition.

For instance, the NYVAC vector, derived by deletion of specific virulence and host-range genes from the Copenhagen strain of vaccinia (Tartaglia et al., 1992) has proven useful as a recombinant vector in eliciting a protective immune response against an expressed foreign antigen. Likewise, the ALVAC vector, a vaccine strain of canarypox virus, has also proven effective as a recombinant viral vaccine vector (Perkus et al., 1995). In non-avian hosts, both these vectors do not productively replicate (with some exceptions as to NYVAC). Since all poxviruses replicate in the cytoplasm and encode most, if not all of the proteins required for viral transcription (Moss 1990), appropriately engineered foreign coding sequences under the control of poxvirus promoters are transcribed and translated in the absence of productive viral replication.

It would be an improvement over the state of the art to provide enhanced vectors, e.g., vectors having enhanced transcription or translation or transcription and translation and/or expression, for instance such vectors which are attenuated; especially since attenuation may raise issues of expression levels and/or persistence, and it would be an advancement to address such issues.

OBJECTS AND SUMMARY OF THE INVENTION

Recent studies on vaccinia replication have revealed certain poxvirus-encoded functions which play a role in the regulation of viral transcription and translation (reviewed in Moss, 1990; Moss, 1992). Some of these vaccinia encoded functions (e.g., E3L, K3L, H4L, and combinations thereof) have now surprisingly been utilized to increase the levels and persistence of gene expression (e.g., foreign gene expression) in vectors (e.g., the NYVAC and ALVAC vectors); and, are exemplary of the inventive vectors and methods.

Objects of the present invention may include at least one of: providing a method for increasing transcription or translation or transcription and translation and/or expression from at least one nucleotide sequence of interest by a vector, such as a coding nucleotide sequence by a vector; a vector having enhanced transcription or translation or transcription and translation; providing a method for preparing a vector having enhanced transcription or translation or transcription and translation and/or expression; providing a method for enhancing transcription or translation or transcription and translation and/or expression from a vector; providing an improved vector, such as poxvirus vectors, e.g., improved NYVAC, ALVAC or TROVAC vectors; and, products therefrom.

The invention thus provides a vector for enhanced expression of at least one first nucleotide sequence. The vector is modified to comprise at least one second nucleotide sequence encoding a transcription factor or translation factor or a transcription factor and a translation factor. The vector also can be modified to comprise the first nucleotide sequence. There is substantially co-temporal or substantially contemporaneous expression from the first and second nucleotide sequences. The expression is in a cell having a particular phenotype, and preferably the expression of the first and second nucleotide sequences is with respect to the phenotype of the cell. Thus, expression of the second nucleotide sequence enhances expression of the first nucleotide sequence by enhancing transcription or translation or transcription and translation.

The first nucleotide sequence can be operably linked to a first promoter and the second nucleotide sequence can be operably linked to a second promoter, and the first and second promoters are preferably functional substantially co-temporally or contemporaneously. Thus, the first and second nucleotide sequences can be at different loci within the vector. The first and second nucleotide sequences also can be at the same locus within the vector, using the first and second promoters; or, by the first nucleotide sequence and the second nucleotide sequence being operably linked to a promoter.

The transcription factor can be of poxvirus origin, e.g., from a vaccinia virus. The transcription factor can be from an open reading frame selected from the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, and combinations thereof. The translation factor can effect inhibition of eIF-2α phosphorylation or inhibition of PKR phosphorylation or otherwise sequester dsRNA which actually increases the concentration required to activate PKR. The translation